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CHEMICAL COMPOSITION OF ESSENTIAL OILS OF S*alvia limbata* FROM TWO DIFFERENT REGIONS IN IRAN AND THEIR BIOLOGICAL ACTIVITIES

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The essential oils and extracts obtained from many plants have recently gained popularity and scientific interest because of their uses in the food, drug, and perfumery industries [1]. Scientists have been interested in biologically active compounds isolated from plant species for the elimination of pathogenic microorganisms because of the resistance that they have built against antibiotics [2]. Plant products could be useful in preserving food storages from contamination [3], whereas the synthetic antioxidants that have been used previously are now toxicologically suspect [4, 5].

Salvia, the largest genus of Lamiaceae. Sixty species of the genus *Salvia* are found in Iran, of which 17 are endemic. *Salvia limbata* C. A. Mey. is a native plant of Iran. Some members of this genus are of economic importance since they have been used as flavoring agents in perfumery and cosmetics. For example, clary sage (*S. sclarea*) is commercially cultivated and its essential oil is widely used as flavoring [6]. Meadow sage (*S. pratensis*) is used in cosmetics and has some medicinal properties [7].

Some of the phenolic compounds or essential oils of plants belonging to this genus have also shown excellent antimicrobial activity [8] and scavenging ability against active oxygen radicals [9], as well as inhibiting lipid peroxidation [10]. Consequently, the corresponding extracts have been used to stabilize fat and fat-containing foods [11]. The antioxidant activity of various species of *Salvia* has been reported previously [12–15].

As far as our literature survey could ascertain, the antimicrobial and antioxidant activities of essential oil and various extracts of *Salvia limbata* have not been published previously, although there are some reports on the essential oil composition [16 – 18] and norsesterterpenes and diterpenes isolated from this species [19, 20].

In the present study, the antimicrobial and antioxidant capacities of the essential oils and various extracts of *S. limbata* from two different regions in Iran (Takab and Mashhade Ardehal) are investigated.

The aerial parts of *S. limbata* C. A. Mey. were collected during the flowering stage in July 2003 from Takab (West Azerbaijan province, SL1) and Mashhade Ardehal (Kashan province, SL2).

The air-dried and ground aerial parts of plants (120 g) were subjected to hydrodistillation for 3 h using a Clevenger type apparatus. The oils were obtained in 0.08% and 0.24% (w/w) yields for the SL1 and SL2 samples, respectively. The essential oils were dried over anhydrous Na_2SO_4 and stored at 4°C until tested and analyzed (Table 1 and 3) [21].

Ten grams of air-dried and powdered plants were extracted with methanol at room temperature for 24 h. After evaporation of the solvent under reduced pressure, the remaining crude materials (M) were suspended in water and extracted with *n*-hexane to yield water soluble (M-W) and water insoluble (M-H) subfractions (Table 2) [22].

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TABLE 1. Chemical Composition of the Essential Oils of *Salvia limbata**, %

Compound	RI[a] (DB-1)	SL1	SL2	Compound	RI[a] (DB-1)	SL1	SL2
α-Thujene	926	0.4	-	α-Gurjunene	1419	0.5	-
α-Pinene[c]	938	24.4	3.8	*trans*-α-Bergamotene	1430	-	0.8
Camphene	950	1.9	0.3	*trans*-Caryophyllene[c]	1432	5.2	9.9
Sabinene	972	3.9	2.9	*trans*-β-Farnesene	1455	-	0.8
β-Pinene[c]	981	21.9	5.8	α-Humulene	1461	0.2	Tr.
β-Myrcene	984	-	0.5	Aromadendrene	1468	0.2	-
α-Phellandrene	1002	0.3	-	γ-Muurolene	1470	-	Tr.
δ-3-Carene	1011	0.3	-	Germacrene D	1489	2.2	3.8
α-Terpinene	1013	0.3	0.4	Bicyclogermacrene	1507	2.6	7.2
p-Cymene[c]	1018	0.4	0.6	γ-Cadinene	1518	0.3	1.5
1,8-Cineole[c]	1029	7.7	9.2	δ-Cadinene	1524	0.8	0.5
γ-Terpinene[c]	1053	0.8	1.1	Spathulenol	1583	1.4	8.1
α-Terpinolene	1085	1.7	5.3	Caryophyllene oxide	1584	0.2	0.6
α-Campholenal	1112	0.2	0.5	Viridiflorol	1599	-	4.0
1-Terpineol	1113	-	0.2	Ledol	1605	0.1	-
trans-Pinocarveol	1132	0.6	1.0	Humulene oxide	1611	-	0.2
Verbenol	1136	0.5	0.7	Cubenol	1616	0.6	1.2
Pinocarvone	1147	0.2	0.5	δ-Cadinol	1636	3.1	0.2
p-Menth-1-en-8-ol	1153	0.2	1.6	γ-Cadinol	1644	-	4.6
Borneol[c]	1158	1.9	2.7	α-Cadinol	1654	-	0.5
p-Cymen-8-ol	1166	0.1	-	β-Eudesmol	1650	0.6	-
4-Terpineol	1171	1.0	3.3	α-Eudesmol	1654	0.2	-
α-Terpineol	1171	0.8	-	Valeranone	1676	-	0.6
Myrtenol	1187	0.3	0.7	α-Bisabolol	1678	-	0.2
trans-Carveol	1205	-	0.1	Leden oxide[b]	1682	-	0.2
Linalyl acetate	1241	-	Tr.	(*Z*,*E*)-Farnesol	1696	-	0.1
Bornyl acetate	1276	2.1	0.5	Hexahydrofarnesyl acetone	1833	-	0.2
α-Terpinenyl acetate	1337	1.0	0.4	Sclareol oxide[b]	1898	0.3	4.3
Eugenol	1338	-	0.1	Neryl linalool	1939	0.1	-
δ-Elemene	1341	0.1	0.5	Hexadecanoic acid	1953	-	Tr.
β-Damascenone	1363	-	0.3	Manoyl oxide	2004	-	Tr.
cis-Jasmone	1376	-	0.3	Phytol	2105	3.9	0.2
α-Copaene	1383	0.4	0.3	Sclareol	2122	-	4.9
β-Bourbonene	1385	Tr.	0.2	Total		96.2	98.7
β-Elemene	1393	0.3	0.3				

*Method of identification: MS, RI; MS (b); MS, RI, Co-1 (c).

[a]RI, retention indices relative to C_6-C_{24} *n*-alkanes.

Tr.: trace (<0.1%).

Chemical Composition of the Essential Oils. The chemical composition of the essential oil of *S. limbata* obtained from two different locations is summarized in Table 1, where all compounds are listed in order of their elution from DB-1 column. As shown, 46 and 56 components representing 96.2% and 98.7% of the total oils obtained from SL1 and SL2 samples were characterized, respectively. α-Pinene (24.4%), β-pinene (21.9%), and 1,8-cineole (7.7%) were the major compounds in SL1, while in SL2, *trans*-caryophyllene (9.9%), 1,8-cineole (9.2%), and spathulenol (8.1%) were found to be the most abundant constituents. Thirty-four compounds were common in both samples; however, in some cases considerable difference in the percentages was observed. For example, α-pinene and β-pinene were found in a total amount of 46.3% in SL1 while they amounted up to 9.6% in SL2. Several compounds were also found in only one of the two samples, of which α-terpineol (0.8%) in SL1 and sclareol (4.9%) and viridiflorol (4.0%) in SL2 were the most outstanding examples.

TABLE 2. Antioxidant Activities of Various Extracts of *S. limbata* against DPPH (IC_{50}) [22]

Extracts	*S. limbata* (SL1)	*S. limbata* (SL2)
Methanolic extract (M)	40.5±0.3	32.3±0.42
Water soluble fraction of methanol extract (M-W)	46.0±0.53	37.0±0.75
Water insoluble fraction of methanol extract (M-H)	100±0.4	95.7±0.59
BHT	25.3±4.3	25.3±4.3

TABLE 3. Antimicrobial Activity of the Oils and Their Main Components of Two Samples of *Salvia limbata*

Test organism	Inhibition zone[a]						MIC[b]				
	SL1	SL2	1,8-cineole	β-pinene	α-pinene	ampicillin	SL1	SL2	1,8-cineole	β-pinene	α-pinene
			10 µL/disk								
Bacillus subtilis	15	10	25	15	10	14.2	7.5	15	0.93	1.87	7.5
Staphylococcus aureus	8	8	15	9	8	13.1	N.t.	N.t.	3.75	15	>15
Staphylococcus epidermidis	19	13	18	12	9	19.0	3.75	7.5	1.87	7.5	15
Enterococcus faecalis	-	8	10	8	-	11.2	N.t.	N.t.	7.5	15	N.t.
Escherichia coli	12	11	20	10	11	12.0	7.5	15	1.87	7.5	15
Klebsiella pneumoniae	-	-	8	-	-	-	N.t.	N.t.	7.5	N.t.	N.t.
Pseudomonas aeruginosa	-	-	-	-	-	9.7	N.t.	N.t.	N.t.	N.t.	N.t.
Candida albicans	14	9	11	-	-	N.t.	20	20	10	N.t.	N.t.
Saccharomyces cerevisiae	11	-	14	-	-	N.t.	>20	N.t.	10	N.t.	N.t.

[a]Diameter of inhibition zones (mm) including diameter of sterile disk (6 mm).
[b]Minimum inhibitory concentration (as mg/mL).
(-), Inactive; (7-14), moderately active; (>14), highly active; N.t.: not tested; SL: *Salvia limbata*.

In a recent paper, the oils of *S. limbata* collected from two areas in eastern Turkey were reported to contain α-pinene (11.2–24.3%), β-pinene (10.0–20.9%), and sabinene (14.6–17.4%) as the major constituents [16]. Mirza et al. identified 26 compounds in the essential oil of *S. limbata* and reported germacrene D (25.7%), linalyl acetate (16.1%), and linalool (17.5%) as the major components [17]. These differences might have been derived from local, climatic, and seasonal factors.

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